```
=> file medline hcaplus biosis biotechds scisearch embase
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 12:51:29 ON 07 DEC 2004

FILE 'HCAPLUS' ENTERED AT 12:51:29 ON 07 DEC 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 12:51:29 ON 07 DEC 2004
Copyright (c) 2004 The Thomson Corporation.

FILE 'BIOTECHDS' ENTERED AT 12:51:29 ON 07 DEC 2004
COPYRIGHT (C) 2004 THE THOMSON CORPORATION

FILE 'SCISEARCH' ENTERED AT 12:51:29 ON 07 DEC 2004
Copyright (c) 2004 The Thomson Corporation.

FILE 'EMBASE' ENTERED AT 12:51:29 ON 07 DEC 2004
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

=> s antifolate therapy and human dihydrofolate reductase
L1            2 ANTIFOLATE THERAPY AND HUMAN DIHYDROFOLATE REDUCTASE

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2            1 DUP REM L1 (1 DUPLICATE REMOVED)

=> d l2 ibib ab

L2   ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2004 ACS on STN DUPLICATE 1
ACCESSION NUMBER:         1995:374749  HCAPLUS
DOCUMENT NUMBER:          122:123105
TITLE:                    Protection of human bone marrow from high dose
                          antifolate therapy using a gene for
                          human dihydrofolate
                          reductase resistant to antifolates
INVENTOR(S):              Bertino, Joseph R.; Gilboa, Eli; Li, Minx-Xia;
                          Schweitzer, Barry I.; Banerjee, Debabrata; Zhao,
                          Shi-Cheng
PATENT ASSIGNEE(S):       Sloan-Kettering Institute for Cancer Research, USA
SOURCE:                   PCT Int. Appl., 209 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.          KIND   DATE        APPLICATION NO.          DATE
     ---------------     ----   --------    --------------------     --------
     WO 9424277           A1    19941027    WO 1994-US4129           19940413
         W:  AU, CA, FI, HU, JP, KR, NO, NZ, RU, US
         RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
     AU 9467047           A1    19941108    AU 1994-67047            19940413
PRIORITY APPLN. INFO.:                      US 1993-49284        A   19930413
                                            WO 1994-US4129       W   19940413
AB   An expression vector carrying the gene for a human antifolate-resistant,
     dihydrofolate reductase is described for use in the protection of bone
     marrow in the course of antifolate therapy. Bone
     marrow cells transformed with this vector are also described for use as
     replacements for hematopoietic stem cells poisoned by antifolate
     therapy. A no. of double-copy retroviral vectors based on the
```

Moloney murine leukemia virus deriv. N2 were constructed. The vectors used one of several mammalian or viral promoters to drive expression of of human or mouse genes for methotrexate-resistant dihydrofolate reductases. These constructs increased the CD50 for methotrexates in animal cell lines by .apprx.2-fold. Irradiated mice transplanted with transgenic bone marrow cells showed prolonged resistance to methotrexate cytotoxicity.

```
=> s pharmaceutical and human dihydrofolate reductase
L3            7 PHARMACEUTICAL AND HUMAN DIHYDROFOLATE REDUCTASE

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4            7 DUP REM L3 (0 DUPLICATES REMOVED)

=> d l4 1-7 ibib ab

L4    ANSWER 1 OF 7  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-18883  BIOTECHDS
TITLE:            High-throughput screening method, useful for identifying
                  pharmaceutical or cosmetic agents, based on comparing
                  effects of test compounds on test and control organisms
                  differing in two selected features;
                     high throughput screening and target gene selection for
                     use in drug screening
AUTHOR:           ESCHRICH D; ENTIAN K; RECKTENWALD J
PATENT ASSIGNEE:  PHENION GMBH and CO KG
PATENT INFO:      DE 10261834 8 Jul 2004
APPLICATION INFO: DE 2002-1061834 20 Dec 2002
PRIORITY INFO:    DE 2002-1061834 20 Dec 2002; DE 2002-1061834 20 Dec 2002
DOCUMENT TYPE:    Patent
LANGUAGE:         German
OTHER SOURCE:     WPI: 2004-518966 [50]
AB    DERWENT ABSTRACT:
      NOVELTY - Screening method for identifying active agents (A) and suitable
      for high throughput systems, is new.
           DETAILED DESCRIPTION - Screening method for identifying active
      agents (A) and suitable for high throughput systems comprises: (a)
      selecting a target organism (TO) that (A) should inhibit; (b) selecting a
      target gene (TG) the gene product of which sholud by deactivated by (A);
      (c) selecting an organism that is to be protected against injury caused
      by TO; (d) selecting a test organism (T1) that contains a test gene (TG1)
      functionally homologous with TG; (e) constructing two test strains of
      (T1) that differ genotypically in just two respects; (f) culturing both
      strains together, treatment with test compound and, on the basis of any
      differences in the growth of the strains, identifying test compounds as
      (A). The two test strains differ in that TG1 in one strain can tolerate a
      higher dose of agent that inactivates TG or its product and by the
      presence of a gene that encodes an easily detectable product that is not
      essential for vitality or proliferative capacity. An INDEPENDENT CLAIM is
      also included for a test kit for identifying (A) by the new method.
           USE - The method is used to identify potential
      pharmaceutical and/or cosmetic agents, e.g. antibiotics;
      cytostatics; or enzyme inhibitors such as inhibitors of HMG-CoA
      reductase, but also contemplated are agents for use against animal and
      plant pathogens.
           ADVANTAGE - The method allows testing of many target genes without
      knowledge of their precise function; provides a very simple read out; by
      mixing target and control organisms together, the total number of wells
      required is reduced by 50%; sensitivity can be adjusted through the
      amount (or ratio) of test strains used; even minimally interfering
      concentrations of active compounds can be measured or detected; the use
      of microorganisms avoids the need for complex extracts; once established,
      the method is quick and inexpensive; only relevant substances generate a
      'hit' (avoiding false positives) and the 'hits' have a high probability
```

of being useful lead compounds.

EXAMPLE - In a screen for dihydrofolate reductase (DHFR) inhibitors, the control strain was Escherichia coli that carried a plasmid containing the human DHFR gene, and the target strain was similar but lacked the plasmid. A substance that inhibited bacterial DHFR should inhibit growth of only the target strain. The control strain also carried a plasmid that expressed green fluorescent protein (GFP). A mixture of both strains was cultured overnight at 37 degrees Centigrade in wells, with various test compounds. Wells that showed increased fluorescence contain more of the GFP-expressing strain, as a result of inhibition of bacterial DHFR and thus of the target strain. (15 pages)

L4 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1998:367920 BIOSIS
DOCUMENT NUMBER: PREV199800367920
TITLE: Mechanism-based inhibition of human folylpolyglutamate synthetase: Design, synthesis, and biochemical characterization of a phosphopeptide mimic of the tetrahedral intermediate.
AUTHOR(S): Tsukamoto, Takashi; Haile, William H.; McGuire, John J.; Coward, James K. [Reprint author]
CORPORATE SOURCE: Dep. Chem. and Medicinal Chem., Univ. Michigan, Ann Arbor, MI 48109-1055, USA
SOURCE: Archives of Biochemistry and Biophysics, (July 1, 1998) Vol. 355, No. 1, pp. 109-118. print.
CODEN: ABBIA4. ISSN: 0003-9861.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Aug 1998
Last Updated on STN: 27 Aug 1998

AB Folylpolyglutamate synthetase (FPGS) catalyzes an ATP-dependent ligation reaction that results in the synthesis of poly(gamma-glutamate) metabolites of folates and some antifolates. We have synthesized and characterized the prototype of a new class of mechanism-based FPGS inhibitor in which a phosphonate moiety mimics the tetrahedral intermediate formed during the ligation reaction. This phosphonate, 4-amino-4-deoxy-10-methyl-pteroyl-L-glutamyl-gamma-(PSI(P(O)(OH)-O))glutarate (4-NH2-10-CH3-Pte-L-Glu-gamma-(PSI(P(O)(OH)-O))glutarate), is not a substrate for human FPGS, but is a linear, competitive inhibitor (Kis = 46 nM) with respect to methotrexate as the variable substrate. Inhibition is not time-dependent and preincubation of FPGS with this phosphonate does not increase the degree of inhibition, suggesting that it is not a slow, tight-binding inhibitor involving a time-dependent isomerization, EI fwdarw EI*. Substructures containing the phosphonate moiety but lacking the pterin are much less inhibitory to FPGS, indicating that a significant portion of the inhibitor binding energy is derived from the pterin moiety, a feature also observed in substrate binding. 4-NH2-10-CH3-Pte-L, Glu-gamma-(PSI(P(O)(OH)-O))glutarate is also an analog of a proposed tetrahedral intermediate in the reaction catalyzed by gamma-glutamyl hydrolase (gamma-GH), another enzyme of importance in controlling folate homeostasis in cells. This intermediate would arise from direct attack of H2O on the dipeptide, 4-NH2-10-CH3-Pte-L-Glu-gamma-Glu. The fact that 4-NH2-10-CH3-Pte-L-Glu-gamma-(PSI(P(O)(OH)-O))glutarate is not an inhibitor of gamma-GH strongly suggests that hydrolysis of poly-gamma-glutamates catalyzed by gamma-GH does not involve the direct attack of water at the scissile amide bond. Methotrexate, its gamma-glutamyl dipeptide metabolite, and 4-NH2-10- CH3-Pte-L-Glu-gamma-(PSI(P(O)(OH)-O)glutarate are equipotent as inhibitors of **human dihydrofolate reductase** (the primary target of methotrexate), but the phosphonate does not significantly inhibit another important folate-dependent enzyme, thymidylate synthase. Thus, the phosphonate moiety in this analog represents an important new lead in the development of FPGS inhibitors.

L4 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1996:81631 BIOSIS
DOCUMENT NUMBER: PREV199698653766
TITLE: Synthesis and biological activity of folic acid and methotrexate analogues containing L-threo-(2S,4S)-4-fluoroglutamic acid and DL-3,3-difluoroglutamic acid.
AUTHOR(S): Hart, Barry P.; Hale, William H.; Licato, Nicholas J.; Bolanowska, Wanda E.; McGuire, John J.; Coward, James K. [Reprint author]
CORPORATE SOURCE: Dep. Chem., University Michigan, Ann Arbor, MI 48109-1055, USA
SOURCE: Journal of Medicinal Chemistry, (1996) Vol. 39, No. 1, pp. 56-65.
CODEN: JMCMAR. ISSN: 0022-2623.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Feb 1996
Last Updated on STN: 10 Jun 1997

AB The stereospecific syntheses of L-threo-gamma-fluoromethotrexate (1t) and L-threo-gamma-fluorofolic acid (3t) are reported. Compounds 1t and 3t have no substrate activity with folylpoly-gamma-glutamate synthetase isolated from CCRF-CEM human leukemia cells, and compound 1t inhibits **human dihydrofolate reductase** at similar levels as methotrexate. The synthesis of DL-3,3-difluoroglutamic acid (6) and its incorporation into DL-beta,beta-difluorofolic acid (4) are also reported. Compound 4 acts as a better substrate for human CCRF-CEM folylpoly-gamma-glutamate synthetase than folic acid (V/K = ca. 7-fold greater). Thus, replacement of the glutamate moiety of methotrexate and folic acid with 4-fluoroglutamic acid and 3,3-difluoroglutamic acid results in folates and antifolates with altered polyglutamylation activity.

L4 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:665544 HCAPLUS
DOCUMENT NUMBER: 119:265544
TITLE: In vivo binding pair pretargeting for site-specific delivery of functional moiety in radioimaging or radiotherapy
INVENTOR(S): Pomato, Nicholas; McCabe, Richard P.; Hawkins, Gregory A.; Brederhorst, Reinhard; Kim, Chong Ho; Vogel, Carl Wilhelm
PATENT ASSIGNEE(S): AKZO N.V., Neth.
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9317707	A1	19930916	WO 1993-US1858	19930303
W: AU, CA, FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9337368	A1	19931005	AU 1993-37368	19930303
AU 663582	B2	19951012		
EP 590109	A1	19940406	EP 1993-906276	19930303
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 06507918	T2	19940908	JP 1993-515830	19930303
ZA 9303035	A	19931209	ZA 1993-3035	19930429
US 5578289	A	19961126	US 1993-140186	19931104
PRIORITY APPLN. INFO.:			US 1992-846453 A2	19920304
			WO 1993-US1858 A	19930303

AB A method for the in vivo targeting of a functional moiety in a patient (e.g. for imaging or therapy) comprises 1st administering a targeting moiety (e.g. antibody) coupled to an enzyme and thereafter administering a

binding partner for the enzyme (e.g. enzyme inhibitor, enzyme substrate) coupled to a functional moiety forming an effector complex (preferably a radiometal complex), whereby the effector complex through the binding partner binds to the enzyme to localize the functional moiety in the target area. Recombinant **human dihydrofolate reductase** was conjugated with antitumor monoclonal antibody (MAb) 16.88 or with anti-human transferrin receptor MAb 5E9C11 via a heterobifunctional crosslinker. Methotrexate (a dihydrofolate reductase inhibitor) analog-DTPA (linked at the .gamma.-carboxyl group of the glutamic acid) was prepd. and chelated with 111In. The chelate bound to target cell-bound MAb-enzyme conjugate.

L4 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:8606 HCAPLUS
DOCUMENT NUMBER: 120:8606
TITLE: Pyrroloquinazoline dihydrofolate reductase inhibitors
INVENTOR(S): Kuyper, Lee Frederick; Jones, Michael Lee; Baccanari, David Patrick
PATENT ASSIGNEE(S): Wellcome Foundation Ltd., UK
SOURCE: Eur. Pat. Appl., 31 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
EP 542497	A1	19930519	EP 1992-310232		19921109
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
WO 9310119	A1	19930527	WO 1992-GB2062		19921109
W: AU, BG, CA, CS, FI, HU, JP, KR, NO, PL, RO, RU, UA, US					
AU 9228944	A1	19930615	AU 1992-28944		19921109
CN 1073175	A	19930616	CN 1992-114385		19921110
ZA 9208661	A	19940511	ZA 1992-8661		19921110
PRIORITY APPLN. INFO.:			GB 1991-23916	A	19911111
			WO 1992-GB2062	A	19921109

OTHER SOURCE(S): MARPAT 120:8606
AB The title compds. I [R1 = H, C1-6 alkyl, C1-4 haloalkyl, C1-4 alkoxy; R2, R3 = (un)substituted C1-4 alkyl, C1-4 alkoxy; R4 = H, C1-4 alkyl; R2CR3 = C5-7 cycloalkyl or cycloalkenyl group], which are inhibitors of dihydrofolate reductase, useful in treatment of immune system disorders (no data), malignant tumors, bacterial infections, protozoal infections (no data) and fungal infections (no data), and which are capable of crossing the blood-brain barrier, are prepd., and **pharmaceutical** formulations contg. I are presented. Thus, I (R1 = R4 = H, R2 = R3 = Et), prepd. from 5-aminoindole hydrochloride in four steps, demonstrated 50% inhibitory concn. of **human dihydrofolate reductase** of <0.1 .times. 10-8M.

L4 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1994:130184 BIOSIS
DOCUMENT NUMBER: PREV199497143184
TITLE: Studies on analogues of classical antifolates bearing the naphthoyl group in place of benzoyl in the side chain.
AUTHOR(S): Piper, James R. [Reprint author]; Johnson, Cheryl A.; Maddry, Joseph A.; Malik, Neeta D.; McGuire, John J.; Otter, Glenys M.; Sirotnak, Francis M.
CORPORATE SOURCE: Organic Chem. Res. Dep., Southern Res. Inst., Birmingham, AL 35255, USA
SOURCE: Journal of Medicinal Chemistry, (1993) Vol. 36, No. 26, pp. 4161-4171.
CODEN: JMCMAR. ISSN: 0022-2623.
DOCUMENT TYPE: Article
LANGUAGE: English

ENTRY DATE: Entered STN: 24 Mar 1994
Last Updated on STN: 18 Nov 1994

AB Analogues of classical antifolates with the 4-aminobenzoyl group replaced by 4-amino-1-naphthoyl were synthesized for study after molecular modeling indicated ample spatial accommodation for the naphthalene ring and even larger groups in models based on reported X-ray crystallographic data describing the binding of methotrexate to **human dihydrofolate reductase** (DHFR). The side-chain precursors, N-(4-amino- and 4-(methylamino)-1-naphthoyl)-L-glutamic acid diethyl eaters, were synthesized, and the 2,4-diamino-substituted heterocyclic groups were attached using several methods. Target compounds included naphthoyl analogues of aminopterin (AMT), methotrexate (MTX), 5-deazaAMT, 5-deazaMTX, 5-methyl-5-deazaAMT, 5-methyl-5-deazaMTX, and 5,8-dideazaAMT. A 5,6,7,8-tetrahydronaphthoyl analogue of 5-deazaAMT was also prepared. None of the naphthoyl analogues showed loss in binding to DHFR compared with the corresponding antifolate bearing the benzoyl group, thus confirming the anticipated bulk tolerance. Only the 5,6,7,8-tetrahydronaphthoyl analogue displayed reduced antifolate effects. Substrate activity toward folylpolyglutamate synthetase was, however, severely compromised. The naphthoyl compounds were transported into L1210 cells 3-6 times more readily than MTX, and despite apparently low levels of intracellular polyglutamylation, each compound was found to be significantly more potent than MTX in inhibiting tumor cell growth in vitro in three lines (L1210, HL60, and S180). The MTX, 5-methyl-5-deazaAMT, and 5-methyl-5-deazaMTX analogues were evaluated in vivo alongside MTX against E0771 mammary adenocarcinoma in mice. All three proved more effective than MTX in retarding the tumor growth. The naphthoyl analogue of 5-deazaAMT strongly inhibited DHFR from Pneumocystis carinii, Toxoplasma gondii, and rat liver giving IC50 (pM) values of 0.53, 2.1, and 1.6 respectively, but this compound did not inhibit in vitro growth of T. gondii, thus indicating lack of transport.

L4 ANSWER 7 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1989:430055 BIOSIS
DOCUMENT NUMBER: PREV198988088313; BA88:88313
TITLE: INHIBITION OF MAMMALIAN FOLYLPOLYGLUTAMATE SYNTHETASE AND **HUMAN DIHYDROFOLATE REDUCTASE** BY 5 8 DIDEAZA ANALOGUES OF FOLIC ACID AND AMINOPTERIN BEARING A TERMINAL L ORNITHINE.
AUTHOR(S): PATIL S A [Reprint author]; SHANE B; FREISHEIM J H; SINGH S K; HYNES J B
CORPORATE SOURCE: DEP PHARM SCI, MED UNIV SC, CHARLESTON, SC 29425, USA
SOURCE: Journal of Medicinal Chemistry, (1989) Vol. 32, No. 7, pp. 1559-1565.
CODEN: JMCMAR. ISSN: 0022-2623.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 19 Sep 1989
Last Updated on STN: 28 Oct 1989

AB Six new 5,8-dideaza analogues of folic acid and aminopterin containing a terminal L-ornithine residue were prepared by using multistep synthetic sequences. Each was evaluated as an inhibitor of hog liver folylpolyglutamate synthetase and **human dihydrofolate reductase**. Structural modifications at positions 2, 4, 5, and 10 were included to help define structure-activity relationships for compounds of this type. The compound N.alpha.-(4-amino-4-deoxy-5-chloro-5,8-dideazapteroyl)-L-ornithine (3f) was identified as the most potent inhibitor of mammalian folypolyglutamate synthetase reported thus far (Ki .simeq. 2 nM). Its 4-oxy counterpart, N.alpha.-(5-chloro-5,8-dideazapteroyl)-L-ornithine, was only 5-fold less inhibitory than 3f toward folylpolyglutamate synetheatase but was found to be a much weaker inhibitor of dihydrofolate reductase than 3f.

```
=> d his

     (FILE 'HOME' ENTERED AT 12:50:41 ON 07 DEC 2004)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT
     12:51:29 ON 07 DEC 2004
L1             2 S ANTIFOLATE THERAPY AND HUMAN DIHYDROFOLATE REDUCTASE
L2             1 DUP REM L1 (1 DUPLICATE REMOVED)
L3             7 S PHARMACEUTICAL AND HUMAN DIHYDROFOLATE REDUCTASE
L4             7 DUP REM L3 (0 DUPLICATES REMOVED)

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY    SESSION
FULL ESTIMATED COST                                   31.01      31.22

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                      ENTRY    SESSION
CA SUBSCRIBER PRICE                                   -2.10      -2.10

STN INTERNATIONAL LOGOFF AT 12:56:25 ON 07 DEC 2004
```